Evaluation of The Efficiency of Date Seeds as a Carrier of PGPR Inoculants Under Different Storage Temperature

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Abstract. Two isolates of Plant Growth Promoting Rhizobacteria were used in this experiment, After confirming that these strains belong to Bacillus megaterium and Pseudomonas fluorescence by using agronomic and morphological characteristics, these strains were grown on slants and transferred to liquid broth (NB) in the rotary shaker to prepare mother (starter) culture which contains 31 x 108 , 37 x 108 CFU.ml-1 for each strain. Date palm seeds were used as a carrier for these strains, which had two types (the seeds of Rutab dates and the seeds of the molasses date), these strains were mixed with 10 g for each type of date palm seeds and then divided into two groups. The first group was stored at 4° c and the second group was stored at 25° c for 120 days. Survival cells were counted for each microbial combination every 30 days of storage. The results showed the ability of the date seeds material of both types to maintain the density of the bacterial inoculum, whether B. megaterium or P.flourescence throughout the storage period of 120 days. The combination of S3 retained the highest population density of the inoculum cells after 120 days of storage, for both storage grades 4 and 25° c recording log 7.37 and 7.93 CFU /g, While the combination S2 recorded the lowest microbial density of the inoculum cells which was log 6.25 and 6.32 cfu/g. The date seed (al-Rutab, molasses) maintained a constant level of decrease in the numbers of live cells of the Pseudomonas fluorescence (S3, S4) at both storage degrees, the percentage of reduction was 22 and 16% respectively at the end of period storage, as for B. megaterium the combination S1 and S2 were recorded 18 and 30%.

1. Introduction

Pgpr is one of the bacterial groups that can be found in the rhizosphere automatically or added in the form of bacterial inoculants, which in turn enhance plant growth through their role in facilitating the readiness of some nutrients or biological control agents for some diseases that may affect the plant or as regulators growth of some environmental stresses in the soil [1]. The biological inoculant can be defined as the composition that can contain one or more types of microorganisms beneficial for plant growth and that ensure the achievement of the desired goal of their use. Therefore, the development of production techniques for microbial inoculants in large quantities with potential infection events is the main issue that must be taken into consideration to promote the optimal and widespread use of biological fertilizers [2]. After a period of adding it to the soil or with the seeds, the bacterial inoculant faces a decrease in the number of bacteria, which contributes to the weakening of the bacterial biomass and makes it difficult to maintain the physiological state of the bacterial inoculant. Therefore, the main role of the inoculant composition is to provide an appropriate environment to ensure that the number of bacteria in the inoculant does not rapidly decrease below the threshold limit (106 -108 cell /plant) during storage or when using it directly in the soil to ensure that the response occurs and achieve the required role for the inoculant in the rhizosphere [3];[4]. There are two main steps to producing the inoculant, the first is to prepare a pure culture of bacteria, and the second step is to mix the culture with the suitable carrier for it [5]. Many materials have been used as carriers for PGPR inoculants, including clay, peat moss, perlite, charcoal, and plant waste [6];[7];[8]. Several characteristics must be available in the materials used as inoculant carriers, including 1- It is environmentally safe 2- Non-toxic for the inoculant used 3- Inexpensive and locally available 4- The pH is close to neutral 5- Good ability to preserve moisture 6- It

contains the necessary nutrients 7- It can be sterilized by one of the sterilization methods [9]. Therefore, this study aimed at preparing bio-fertilizer from PGPR bacteria, selecting a suitable carrier for it, and testing the carrier efficiency in maintaining the bacterial number within the appropriate limits during the storage period.

2-Materials and Methods

2.1.Preparing bacterial isolates

Two isolates were obtained for B. megaterium and Pseudomonas fluorescence, which were isolated from soil. The phenotypic and microscopic characteristics of isolates growing on the culture media of each species were studied in terms of colony shape, color by the naked eye, and nature of their spread over the agricultural medium, and their ability to secrete pigments with age and their interaction with Gram stain [10].

2.2.Preparation of primary culture from bacterial isolates

Transfer a portion of each bacterial culture to 250 mL flasks containing 100 mL of sterile NB (nutrient broth medium) and incubated in a vibrating incubator at 28 ° C ± 2 for 4 days [11].

2.3.Preparing the carrier

The date seeds were selected as a carrier in this study (with two stages of maturity, the rutab and the molasses date seeds) and some treatments were performed on them before mixing with the bacterial culture.

- Washing and cleaning the date seeds well with water and removing impurities from them.
- Drying by air
- The date seeds were ground and crushed to obtain consistent sizes by passing them through a sieve with holes in the diameter of 2 mm.
- Put 10 grams of each type of date seeds in 100 ml glass bottles that can be sterilized, and 0.5 g of CaCO3 was added to the pH equation and put in an autoclave at 121 ° C for 15 minutes and the process was repeated the next day to ensure complete sterilization.
- Add sterile distilled water to the bottles to reach a humidity of 50-60 %[11].
- Mix the bacterial isolates with the carrier

Add 2 ml from each bacterial culture, one ml of which contains 31 x 108 and 37 x 108 CFU/ml to the bottles containing the carrier. Then, the bottles were divided into two groups; the first was stored at 25 ° C± 2 and the second in the refrigerator at 4 ° C ± 2 for 120 days. Do the bacterial count every 30 days by Plate Count Technique [12]

The following symbols are given according to the type of inoculant and the carrier:

S1 B. megaterium inoculum loaded on rutab date seed
S2 B. megaterium inoculum loaded on a molasses date seed
S3 P.flourecence inoculum loaded on rutab date seed
S4 P.flourecence inoculum loaded on a molasses date seed

3. Results and discussion

Through Figures 1 and 2, we note the ability of the date seeds of both types to maintain the numerical density of the bacterial inoculum, whether B.megaterium or P.flourecence throughout the storage period of 120 days. Figure No. 1, which represents storage at a temperature of 4° C, shows that the numerical density of the two types of bacterial inoculum decreased during the storage period without recording a significant difference. The S3 combination retained the highest density of the inoculum cells after 120 days of storage, recording a log of 7.37 CFU / g, followed by the S4 and S1 which are recorded log 7.26,7.23 CFU /g, respectively, while the combination S2 recorded the lowest population density of the inoculum after the expiration of the storage period, and it was log 6.25 CFU / g. As for the P.flourecence bacteria inoculum, the numbers of survival cells in the inoculum S4 and S3 did not change, loaded on the seeds of dates of both types (al-Rutab and dates molasses), the results were close between them, and during the storage period they maintained a constant level of reduction of about 8% in the number of live

cells if the reading after the first 30-day storage period was log 9.53, 9.55 CFU / g, respectively, and after 60 days of storage, it was log 8.75, 8.89 CFU / g, and at the end of the storage period, that is, after 120 days, log 7.37, 7.26 CFU / g, respectively, was recorded. It reached 22% from beginning to end of storage. . The numbers of B. megaterium bacteria differed in the date seeds of two types S1 and S2 compared to the P.flourecence inoculum, as it was recorded after 30 days of storage log 8.27, 8.94 CFU / g, a decrease of 4%. The number of bacteria differed after 90 days of storage if log 7.74. , 6.79 CFU / g, and this variation continued until the end of the storage period, it was log 7.23, 6.25 CFU / g were recorded, with a decrease of 12.5% in the S1 combination and 30% in the S2 combination. The combination S3 recorded the highest population density of inoculant cells when stored at 25 ° C and after 120 days of storage if log 7.93 CFU / g, followed by S4 and S1 combinations with a numerical density of log 7.13.6.82 CFU / g, respectively, while the S2 combination recorded the lowest number density of Inoculum cells reached log 6.32 CFU / g Fig. 2. The numbers of live cells of P.flourecence in the combinations of S3 and S4 decreased by 2% at the first 30 days of storage and for both types of the carrier material (dates al-rutab and molasses seed), while the decrease ranged from 6-9% after 60 days of storage and stabilized at the end of Storage period (after 120 days) to 16% and 12% for S3 and S4 blends, respectively. The numbers of B. megaterium bacteria did not differ much compared to P.flourecence, as it recorded a relative decrease of 0.7 and 3% in the S1 and S2 combinations in the first 30 days of storage and this decrease increased after 60 days to ranges between 10-12% at the end of the storage period, the decrease is 18%. The percentage of live cells of the PGPR inoculant in the date seed material varied in its two types (rutab and molasses) during the storage period and at the storage levels 4° and 25° c, but in most combinations it remained within the threshold limit that guarantees the bacteria to settle in the rhizosphere and perform its vital functions that positively reflect on promoting growth the plant. The ability of the date nucleus material to maintain the density of live cells of the PGPR inoculant may be due to its good content of nutrients, oleic acids, proteins, fats, fibers, and some trace elements such as iron, manganese, and sulfur, in addition to the good content of moisture[13];[14] which promotes the growth and reproduction of bacteria cells throughout the storage period These results converged with [15] if it indicated the good role of the date seeds in maintaining the population density of Rhizobia inoculant cells after a month of storage at a temperature of 4 ° C**.** [16] confirmed the suitability of date seeds as a carrier of Azospirilum bacteria and its role in maintaining a high population density in the biological inoculant, which reflected positively on the studied characteristics in the field.

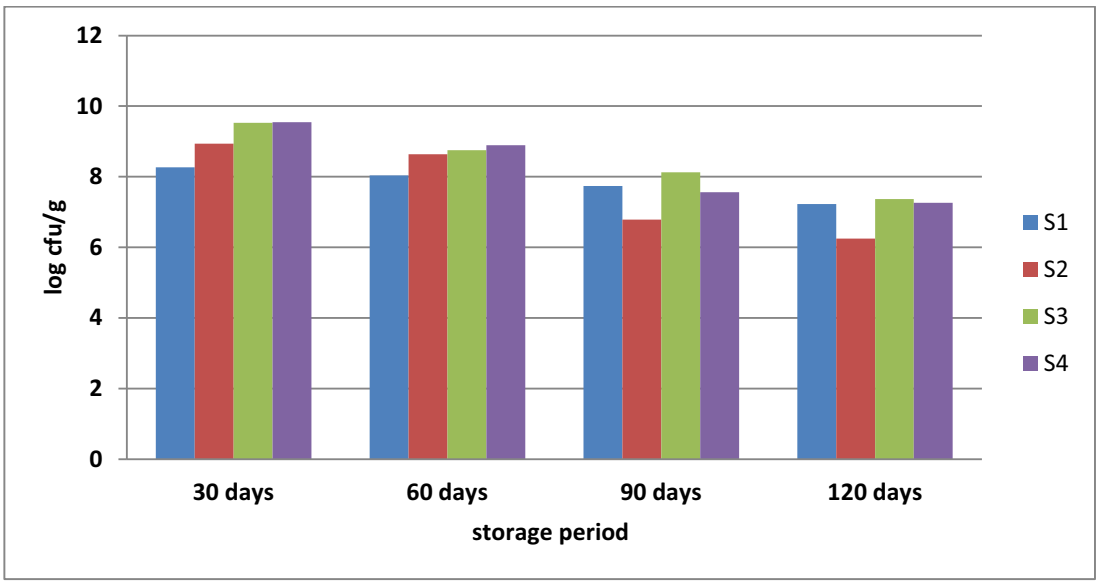

Figure 1. Population density of PGPR inoculants on date palm seeds, stored at4 ° c

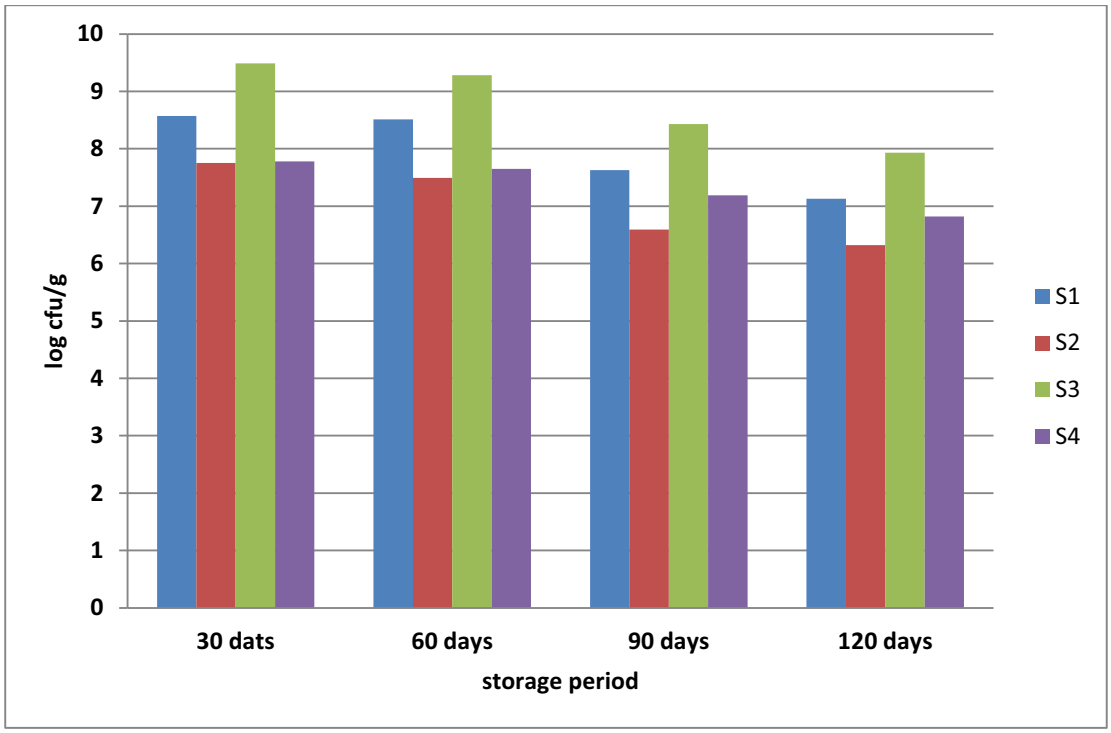

Figure 2. Population density of PGPR inoculants on date palm seeds, stored at 25° c

4. Conclusion

The date palm seeds (al-Rutab and dates molasses) were a suitable carrier of PGPR inoculants when stored at a different temperature if the population density of the bacteria in the inoculant was maintained within the required limit to establish a settlement in the soil when using it.

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